

Mass. Inst. of Tech. Code 1  
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- (1) Paper I: K. Biemann, J. Seibl and F. Gapp, J. Am. Chem. Soc., 83, 3795 (1961).
- (2) This investigation was supported by a grant from the National Aeronautics and Space Administration (NsG 211-62). We would like to thank Mr. M. Munroe for invaluable help with the instrumentation.

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Sir:

In our efforts to extend the applicability of mass spectrometry to organic molecules of extremely low volatility, we have been able to determine the mass spectra of amino acids without prior conversion to more volatile derivatives required for our earlier work.<sup>3</sup> Using the same technique, which had made it possible to obtain mass spectra of

- (3) K. Biemann, J. Seibl and F. Gapp, Biochem. Biophys. Res. Commun. 1, 307 (1959).

nucleosides<sup>4</sup>, volatilization of the sample directly into the ion source and close to the

- (4) K. Biemann and J. A. McCloskey, J. Am. Chem. Soc., 84, 2005 (1962).

ionizing electron beam gave excellent mass spectra of free amino acids and even their hydrochlorides.<sup>5</sup>

- (5) W. L. Baun and D. W. Fischer [Anal. Chem., 34, 294 (1962)] reported the introduction of free amino acids into a spark source mass spectrometer. Our spectrum of lysine hydrochloride (Fig. 1b) indicates that the much more gentle conditions of sublimation of the sample at relatively low temperatures into an electron beam of 70 ev as contrasted to sparking at high frequency with 100 Kv lead to spectra which are much more characteristic of the original molecule.

These spectra<sup>6</sup> (determined with samples ranging from 0.25 - 10  $\mu$ g.) were quite

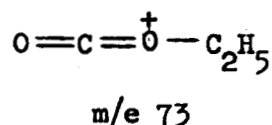
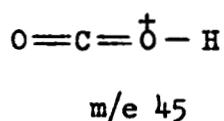
- (6) For experimental conditions see footnote 6 in reference 4. Samples were vaporized at temperatures between 80° and 200°.

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similar to those of the corresponding ethyl esters<sup>1</sup>, indicating that free amino acids, although in the solid state present as zwitterions, exist in the gas phase as the undissociated amino carboxylic acids or possibly hydrogen-bonded forms thereof. It will, therefore, suffice to discuss only those peaks not present as such in the mass spectra of the amino acid ethyl esters.

The presence of the free carboxyl group instead of carbethoxy leads to a shift of mass 28 units in certain peaks (e.g., m/e 102 in ethyl esters vs. m/e 74 in the acids). Furthermore, peaks due to the elimination of a group other than the acid moiety will be found 28 mass units lower in the free acids. There seem to be only two new modes of fragmentation: First, there is always found a peak of significant intensity at m/e 45 corresponding to the carboxyl group while the corresponding one at m/e 73 in ethyl esters is absent. This we attribute to the stability of a positively charged carboxyl fragment which is equivalent to a protonated carbon dioxide molecule while the corresponding carbethoxy ion of mass 73 is energetically less favored.



The second difference is found in the presence of a peak at m/e 75 in the spectra of a number of amino acids such as valine, the leucines (particularly isoleucine), methionine, serine, cysteine, and lysine, for example, i.e., all those containing a hydrogen atom in a  $\gamma$ -position with respect to the carbonyl group. This rearrangement is well known<sup>7</sup> for fatty acids and their esters, but is not observed in  $\alpha$ -amino esters,

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(7) F. W. McLafferty, Anal. Chem., 31, 82 (1959).

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because of the availability of the free electron pair on nitrogen<sup>1</sup>. In the free acids hydrogen bonding seems to decrease this effect, thus favoring this rearrangement which is very sensitive to the electron density at the atom attached to C <sub>$\alpha$</sub> .<sup>8</sup>

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(8) For a detailed discussion of the interpretation of mass spectra see K. Biemann, "Mass Spectrometry," McGraw-Hill, New York, N. Y., 1962, Chap. 3.

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The mass spectrum of methionine (Fig. 1a) illustrates the similarity to that of the ester.<sup>1</sup> The peaks at mass 74, 101, 132, and 149 (mol. wt.) are those which occur 28 mass units higher in the ethyl ester. Fragments of mass 75, 57, and 45 correspond to the additional fragmentation modes discussed above; (m/e 75 is only partly due to  $\text{CH}_3\text{-S-CH}_2\text{-CH}_2^+$ , as evidenced by deuteration experiments).

It is thus possible to interpret the mass spectra of free amino acids based on the behavior of amino esters under electron impact as discussed previously.<sup>1</sup> Glutamic acid appears to dehydrate to pyroglutamic acid prior to sublimation, but it is worth noting that hydroxyamino acids such as serine and hydroxyproline vaporize without decomposition.

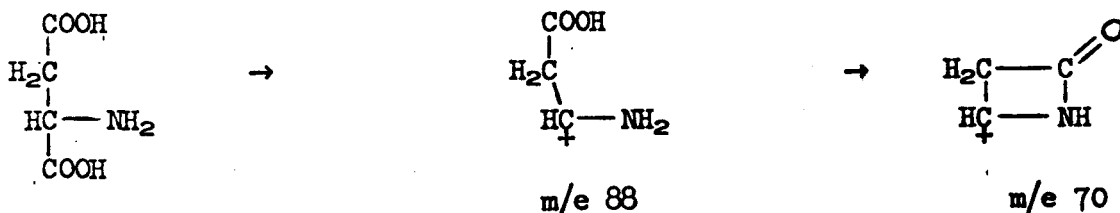
The mechanism of fragmentation has been corroborated by the mass spectra of  $\text{N}^{15}$ -labeled amino acids. The simplicity with which the mass spectra of N,O-perdeuterio derivatives can be obtained<sup>11</sup> made it possible to determine these spectra for many of the amino acids substantiating some of the mechanisms discussed above and resolving some of the ambiguities discussed earlier.<sup>1</sup> For example, the peak at m/e 70 in the mass spectrum of aspartic acid is at m/e 71 in the deuterated sample. Thus elimination

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(11) For experimental details see footnote 9 in reference 4.

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of  $\text{H}_2\text{O}$  ( $\text{C}_2\text{H}_5\text{OH}$  in the case of the ethyl ester) involves a hydrogen atom of the amino group under formation of a cyclic rather than a ketene ion.



Even salts are amenable to this technique if both the corresponding base and acid are sufficiently volatile and thermally stable at the temperature of their dissociation.<sup>12</sup> The resulting mass spectrum shows contributions of the free base

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(12) For example, picrates of organic bases such as alkaloids give the mass spectrum of the free base plus the peaks due to picric acid.

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and the free acid superimposed. Fig. 1b is the mass spectrum obtained from lysine monohydrochloride. The peaks in the region of  $m/e$  35 through 39 in Fig. 1b represent hydrochloric acid while the rest of the spectrum is due to free lysine which again illustrates the similarity to the spectrum of its ethyl ester<sup>2</sup>. Histidine dihydrochloride and cysteine hydrochloride also give rise to very good spectra.

The simplicity, speed, and sensitivity of the method described should make it a valuable tool for the identification and characterization of extremely small amounts of amino acids.

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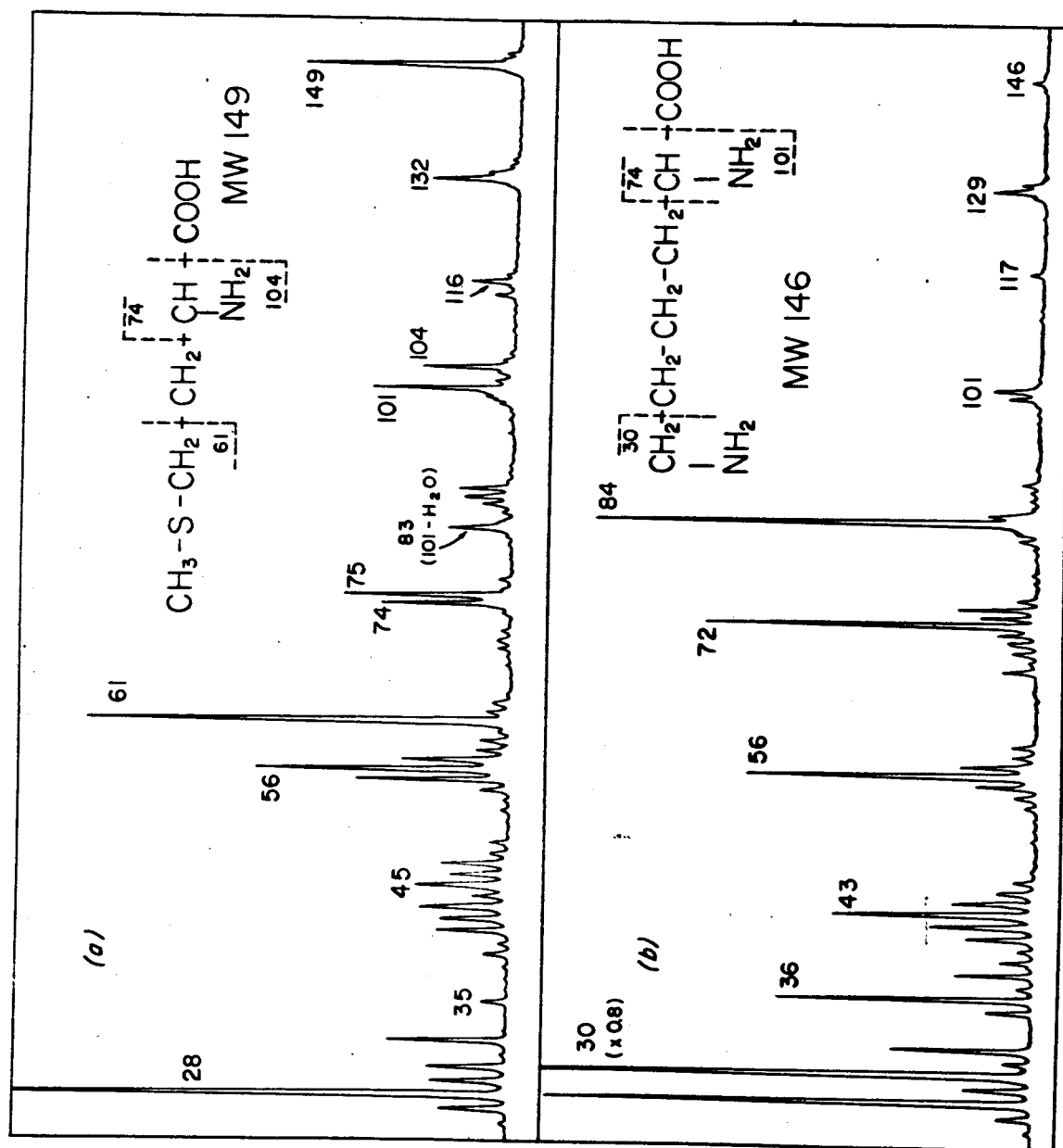


Fig. 1